Inhibition of Sodium-GlucoseCotransporter 2 with dapagliflozin in Han: SPRD rats with polycystic kidney disease

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Abstract: BACKGROUND/AIMS Dapagliflozin (DAPA) is a selective inhibitor of the sodium-glucose cotransporter 2 (SGLT2) which induces glucosuria and osmotic diuresis. The therapeutic effect of DAPA in progressing stages of polycystic kidney disease (PKD) has not been studied. METHODS We examined the effect of DAPA in the Han: SPRD rat model of PKD. DAPA (10 mg/kg/day) or vehicle (VEH) was administered orally via gavage to 5 week old male Han: SPRD (Cy/+) or control (+/+) rats (n = 8-9 per group) for 5 weeks. Blood and urine were collected at baseline and after 2.5 and 5 weeks of treatment to assess renal function and albuminuria. At the end of the treatment, rats were sacrificed and kidneys were excised for histological analysis. RESULTS After 5 weeks of treatment, DAPA-treated Cy/+ and +/+ rats exhibited significantly higher glucosuria, water intake and urine output than VEH-treated rats. DAPA-treated Cy/+ rats also exhibited significantly higher clearances for creatinine and BUN and less albuminuria than VEH-treated Cy/+ rats. DAPA treatment for 5 weeks resulted in a significant increase of the kidney weight in Cy/+ rats but no change in cyst growth. The degree of tubular epithelial cell proliferation, macrophage infiltration and interstitial fibrosis was also similar in DAPA-and VEH-treated Cy/+ rats. CONCLUSION The induction of glucosuria with the SGLT2-specific inhibitor DAPA was associated with improved renal function and decreased albuminuria, but had no effect on cyst growth in Cy/+ rats. Overall the beneficial effects of DAPA in this PKD model were weaker than the previously described effects of the combined SGLT1/2 inhibitor phlorizin.

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Original Paper

Inhibition of Sodium-Glucose Cotransporter 2 with Dapagliflozin in Han: SPRD Rats with Polycystic Kidney Disease

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Key Words
Cyst • Dapagliflozin • Glucosuria • Polycystic kidney disease (PKD) • Sodium glucose cotransporter (SGLT)

Abstract
Background/Aims: Dapagliflozin (DAPA) is a selective inhibitor of the sodium-glucose cotransporter 2 (SGLT2) which induces glucosuria and osmotic diuresis. The therapeutic effect of DAPA in progressing stages of polycystic kidney disease (PKD) has not been studied. ***Methods:*** We examined the effect of DAPA in the Han: SPRD rat model of PKD. DAPA (10 mg/kg/day) or vehicle (VEH) was administered orally via gavage to 5 week old male Han: SPRD (Cy/+) or control (+/+) rats (n = 8-9 per group) for 5 weeks. Blood and urine were collected at baseline and after 2.5 and 5 weeks of treatment to assess renal function and albuminuria. At the end of the treatment, rats were sacrificed and kidneys were excised for histological analysis. ***Results:*** After 5 weeks of treatment, DAPA-treated Cy/+ and +/+ rats exhibited significantly higher glucosuria, water intake and urine output than VEH-treated rats. DAPA-treated Cy/+ rats also exhibited significantly higher clearances for creatinine and BUN and less albuminuria than VEH-treated Cy/+ rats. DAPA treatment for 5 weeks resulted in a significant increase of the kidney weight in Cy/+ rats but no change in cyst growth. The degree of tubular epithelial cell proliferation, macrophage infiltration and interstitial fibrosis was also similar in DAPA- and VEH-treated Cy/+ rats. ***Conclusion:*** The induction of glucosuria with the SGLT2-specific inhibitor DAPA was associated with improved renal function and decreased albuminuria, but had no effect on cyst growth in Cy/+ rats. Overall the beneficial effects of DAPA in this PKD model were weaker than the previously described effects of the combined SGLT1/2 inhibitor phlorizin.

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Introduction

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common form of cystic renal diseases, affecting all ethnic groups worldwide, with an incidence of 1:400-1:1.000 [1, 2]. The disease is caused by a mutation in the *PKD1* or the *PKD2* genes that encode for the proteins polycystin-1 (PC1) and polycystin-2 (PC2) [3]. Altered function and/or a decrease of PC1 or PC2 levels below a certain threshold lead to slow and continuous development of fluid-filled cysts [4], resulting in bilateral renal enlargement and end-stage renal disease (ESRD) in approximately half of the patients [5].

Since ADPKD is relentlessly progressing, effective and specific long-term therapies are needed to halt disease progression. Several drugs are being tested in clinical trials, and some are emerging to be effective in selected patients [6]. Tolvaptan, in particular, is an aquaretic drug which inhibits the vasopressin V_2 receptor in the distal nephron and causes a massive increase in diuresis, thereby suppressing the renal cAMP production and its stimulatory effect on cyst growth [7].

The sodium-glucose cotransporters (SGLTs) are a family of membrane proteins which are involved in the transepithelial transport of glucose in the kidneys and the gut. Within the kidney, SGLT1 is located in the S3 segment of the proximal renal tubule, whereas SGLT2 is found in the S1 segment [8]. SGLT1 is a low-capacity, high-affinity sodium-glucose cotransporter, responsible for 10% of glucose reabsorption, whereas SGLT2 is a high-capacity, low-affinity transporter which mediates 90% of glucose reabsorption [9]. The pharmacological inhibition of SGLTs leads to glucosuria which is accompanied by a constant increase in diuresis. In analogy to the tolvaptan-induced increase in diuresis which has beneficial effects in PKD we speculated that the increase in diuresis which is caused by SGLT inhibitors could also decrease cyst growth in PKD. In a previous study, we have already demonstrated that phlorizin, a dual SGLT1/2 inhibitor, reduced cyst growth and slowed the decline of renal function in the Han: SPRD rat model of PKD [10]. The inhibition of SGLTs was associated with a decrease in ERK1/2 phosphorylation and tubular epithelial cell (TEC) proliferation in the polycystic kidneys.

Dapagliflozin (DAPA) is a selective SGLT2 inhibitor that is clinically used to control glycemia in patients with type 2 diabetes mellitus [11]. DAPA is well absorbed when given by the oral route, whereas phlorizin needs to be applied parenterally since it is only minimally absorbed when applied orally [12]. By causing glucosuria, DAPA effectively corrects hyperglycemia and reduces hemoglobin A_{1c} in patients with type 2 diabetes mellitus [13]. We hypothesized that - similar to phlorizin - DAPA might also have a therapeutic effect in PKD. The purpose of the present study was therefore to assess whether DAPA can reduce cyst growth and improve renal function in heterozygous (Cy/+) Han: SPRD rats.

Materials and Methods

Animals

The Han: SPRD rat colony was established in our animal facility from a litter which was obtained from the Rat Resource and Research Center (Columbia, MO, USA). Heterozygous cystic (Cy/+) and wild-type normal (+/+) rats were used in this study. Only male rats were used since cysts develop more rapidly in male than in female rats. The regulatory commission for animal studies, a local government agency, approved the study protocol. Rats had free access to tap water and standard rat diet.

Study design

Treatment was started in 5-week old male Cy/+ and wild-type +/+ control Han:SPRD rats. Groups of 8-9 rats were used. DAPA was dissolved in 45% propylene glycol + 45% H_2O + 10% ethanol and was

given daily by gavage at a dose of 10 mg/kg for 5 weeks. Control rats were treated with vehicle (45% propylene glycol + 45% H_2O + 10% ethanol) alone. Blood and 24 h urines were collected from each animal at baseline (before treatment), and after 2.5 and 5 weeks of treatment. Blood was taken from the sublingual vein, centrifuged and the plasma collected. Urines were collected in metabolic cages over a 24 h period. All samples were stored at -80°C prior to further examination. In the plasma glucose, creatinine, blood urea nitrogen (BUN), sodium and chloride concentrations were determined, and the urine was tested for glucose, creatinine and BUN concentrations, using standard clinical chemistry methods. After 5 weeks of treatment, rats were euthanatized with isoflurane (Attane, Piramal Healthcare Limited, India) and both kidneys were excised, decapsulated, weighed and fixed in 10% buffered formalin for 2 days for histological analysis.

Urine albumin determination

A rat albumin ELISA kit was used for the measurement of urine albumin concentration (GenWay, San Diego, California). The urine samples were diluted 500 times in the diluent solution. Albumin standard or urine sample were added to the pre-coated plates (100 µl/well). Plates were incubated for 30 min at room temperature and washed 4 times thereafter with phosphate-buffered saline (PBS). Plates were then incubated with horseradish peroxidase-conjugated anti-albumin solution for 30 min at room temperature in the dark, and washed again 4 times. Then 100 µl 3,3′,5,5′-tetramethylbenzidine substrate solution was added into each well, and plates were incubated in the dark for 10 min at room temperature. Then 100 µl stop solution was added, mixed well, and the absorbance at 450 nm was determined using a Tecan ELISA reader (Tecan Group Ltd, Männedorf, Switzerland).

Histology and immunohistology

One kidney was longitudinally cut in half and approximately 2 mm thick slices were embedded in paraffin wax. Consecutive sections (3-5 µm) were prepared and stained with hematoxylin-eosin, the PAS reaction and Gomori's Blue Trichrome using the Artisan Link stainer (Dako, Baar, Switzerland).

Immunohistology was used to stain for Ki67-positive (proliferating) TEC and CD68-positive infiltrating macrophages, using a Dako Autostainer (Dako) and the streptavidin horseradish peroxidase (HRP) method. After deparaffinization and rehydration, sections were incubated in citrate buffer (pH 6.0) for 20 min at 95°C in a pressure cooker. After washing in TBS-Tween, slides were incubated for 60 min at room temperature with the primary antibodies (mouse anti-rat Ki67 [clone MIB-5, Dako], mouse anti-rat CD68 [clone ED1, AbD Serotec, Kidlington, UK]) at a dilution of 1:10 and 1:500 in dilution buffer (Dako), respectively. This was followed by incubation with the secondary antibody for 15 min, the peroxidase blocking solution for 10 min, and the streptavidin HRP for 15 min. After washing, the reaction was visualized by incubation with 3, 3'-diaminobenzidine for 10 min, followed by counterstaining with hemalaun (Merck, Darmstadt, Germany).

Histomorphometry

PAS-stained sections were subjected to cyst index analysis, using the HistoQuant image analysis software (3DHISTECH Kft., Budapest, Hungary) to assess the cortical cystic area (CCA) within the entire cortex (total area, TA). The cyst index was calculated as CCA/TA*100. We counted the total number of cysts detected in 2 full longitudinal sections of each kidney and then averaged the total number of cysts. We calculated the area of each cyst, then counted and arranged them by size. For each section stained for the expression of Ki67, the total number of Ki67-positive nuclei was counted, from which an average number of Ki67-positive nuclei per mm^2 of tissue was calculated. For each section stained for the expression of CD68, the total number of CD68-positive cells was counted, from which an average number of CD68-positive nuclei per mm^2 of tissue was calculated. The extent of fibrosis was determined on Gomori's Blue Trichrome stained sections by measuring the collagen stained area in mm^2 and dividing it by the total area of tissue in mm^2.

Statistical analysis

Data are presented as means ± SD. Statistical differences between treatment groups were assessed by the unpaired two-tailed *t*-test using Graph Pad Prism version 5.0 (Graph Pad, San Diego, CA, USA). $P < 0.05$ was considered to be statistically significant.

Results

DAPA induced glucosuria and increased diuresis

Treatment with DAPA effectively induced glucosuria and increased the urine output in Cy/+rats (Table 1). Alongside the increased diuresis, an increase in water intake was observed with DAPA treatment. The body weight gain was not altered with DAPA in Cy/+. A mild increase in urine Na^+ and Cl^- was seen upon treatment with DAPA in Cy/+ (Table 1).

DAPA preserved the decline of the creatinine and BUN clearances in Cy/+ rats

With increasing age, the clearances for creatinine and BUN increased in VEH- and DAPA-treated +/+ rats to a similar level (Table 2). In VEH-treated Cy/+ rats, creatinine and BUN clearances decreased during the 5-week treatment phase, in parallel with the development of renal cysts. In contrast, DAPA treatment in Cy/+ rats led to preservation of the creatinine and BUN clearances at 2.5 and 5 weeks of treatment (Table 3).

DAPA reduced albuminuria in Cy/+ rats

Albuminuria increased significantly in VEH-treated Cy/+ rats between baseline and week 5. However, DAPA-treated Cy/+ rats showed a significantly lower urine albumin excretion than the VEH-treated rats after 5 weeks of treatment (Table 1).

Table 1. Effects of DAPA on body weight, diuresis, and fluid and electrolyte parameters in Cy/+ rats. Results are expressed as mean ± SD. Comparisons are made between DAPA and VEH groups at the same time point. Abbreviations: VEH, vehicle; DAPA, dapagliflozin; P, plasma; U, urine. *$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$ when comparing DAPA vs. VEH at each time point

	Baseline		2.5 weeks			5 weeks		
	Cy/+ VEH	Cy/+ DAPA	Cy/+ VEH	Cy/+ DAPA		Cy/+ VEH	Cy/+ DAPA	
Number of animals (n)	9	9	9	9		9	9	
Body weight (g)	152.1 ± 12.9	174.6 ± 42.3	278.2 ± 18.9	278.7 ± 31.6		340.5 ± 26.1	341.0 ± 30.9	
Urine volume (ml/day)	10.2 ± 2.5	10.4 ± 7.1	18.2 ± 6.6	35.9 ± 8.4	**	27.5 ± 5.4	42.7 ± 7.0	***
Water intake (ml/day)	30.0 ± 4.4	29.3 ± 8.4	44.2 ± 6.3	62.9 ± 8.1	***	55.6 ± 10.8	75.7 ± 11.0	**
P glucose (mmol/l)	9.0 ± 0.9	9.9 ± 0.6	9.9 ± 1.3	10.2 ± 1.8		13.9 ± 3.2	12.8 ± 2.5	
P Na^+(mmol/l)	139.8 ± 1.3	143.0 ± 4.0	136.8 ± 14.6	139.6 ± 11.1		142.1 ± 1.5	143.4 ± 6.8	
P Cl^- (mmol/l)	103.0 ± 1.0	100.6 ± 3.8	98.1 ± 12.3	96.3 ± 12.0		99.9 ± 6.2	96.9 ± 6.2	
U glucose (mmol/day)	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.3	11.8 ± 3.4	***	0.2 ± 0.0	15.8 ± 4.5	***
U Na^+ (mmol/day)	0.9 ± 0.1	1.2 ± 0.9	1.1 ± 0.2	1.3 ± 0.3		1.3 ± 0.4	1.7 ± 0.3	*
U Cl^- (mmol/day)	1.6 ± 0.3	2.0 ± 1.4	1.8 ± 0.4	2.0 ± 0.5		1.9 ± 0.4	2.5 ± 0.4	*
U albumin (μg/day)	0.1 ± 0.0	0.1 ± 0.1	0.4 ± 0.1	0.5 ± 0.3		3.8 ± 0.9	2.2 ± 0.4	**

Table 2. Effect of DAPA on parameters of renal function in +/+ rats. Results are expressed as mean ± SD. Comparisons are made between DAPA and VEH groups at the same time point. Abbreviations: BUN, blood urea nitrogen; VEH, vehicle; DAPA, dapagliflozin; P, plasma; U, urine. *$P < 0.05$, ***$P < 0.001$ when comparing DAPA vs. VEH at each time point

	Baseline		2.5 weeks			5 weeks		
	+/+ VEH	+/+ DAPA	+/+ VEH	+/+ DAPA		+/+ VEH	+/+ DAPA	
Number of animals (n)	8	9	8	9		8	9	
P BUN (urea) (mg/dl)	17.6 ± 2.1	15.5 ± 1.8	17.1 ± 1.9	23.0 ± 3.4	***	18.7 ± 1.2	28.0 ± 12.1	*
P creatinine (mg/dl)	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.1		0.4 ± 0.0	0.4 ± 0.1	
BUN clearance (ml/min)	0.6 ± 0.1	0.8 ± 0.3	0.9 ± 0.1	0.9 ± 0.1		1.1 ± 0.1	1.2 ± 0.5	
Creatinine clearance (ml/min)	1.7 ± 0.3	1.2 ± 0.8	2.2 ± 0.2	2.1 ± 0.5		2.6 ± 0.3	2.9 ± 0.1	

Table 3. Effect of DAPA on parameters of renal function in Cy/+ rats. Results are expressed as mean ± SD. Comparisons are made between DAPA and VEH groups at the same time point. Abbreviations: BUN, blood urea nitrogen; VEH, vehicle; DAPA, dapagliflozin; P, plasma; U, urine. ** $P < 0.01$, *** $P < 0.001$ when comparing DAPA vs. VEH at each time point

	Baseline		2.5 weeks		5 weeks		
	Cy/+ VEH	Cy/+ DAPA	Cy/+ VEH	Cy/+ DAPA	Cy/+ VEH	Cy/+ DAPA	
Number of animals (n)	9	9	9	9	9	9	
P BUN(urea) (mg/dl)	23.2 ± 11.5	14.8 ± 4.1	25.5 ± 7.8	25.8 ± 4.0	41.7 ± 6.9	32.8 ± 11.6	
P creatinine (mg/dl)	0.3 ± 0.1	0.2 ± 0.2	0.4 ± 0.1	0.4 ± 0.0	0.6 ± 0.1	0.4 ± 0.0	**
BUN clearance (ml/min)	0.6 ± 0.2	0.9 ± 0.5	0.6 ± 0.1	0.7 ± 0.3	0.5 ± 0.1	0.9 ± 0.3	**
Creatinine clearance (ml/min)	1.9 ± 0.3	1.7 ± 1.0	1.8 ± 0.5	2.0 ± 0.3	1.5 ± 0.5	2.3 ± 0.2	***

Table 4. Effect of DAPA on kidney weight in 10-week old +/+ and Cy/+ rats after 5 weeks of treatment. Results are expressed as mean ± SD. Comparisons are made between DAPA and VEH groups at the same time point. Abbreviations: VEH, vehicle; DAPA, dapagliflozin; 2KW, two-kidneys weight; BW, body weight. ** $P < 0.01$, ***$P < 0.001$ when comparing DAPA vs. VEH at each time point

	+/+ VEH	+/+ DAPA		Cy/+ VEH	Cy/+ DAPA	
Number of animals (n)	8	9		9	9	
Kidney Weight (g)	2.3 ± 0.2	3.1 ± 0.3	***	6.8 ± 0.9	8.0 ± 1.3	
2KW/BW (%)	0.7 ± 0.0	0.9 ± 0.1	**	2.0 ± 0.2	2.3 ± 0.2	**

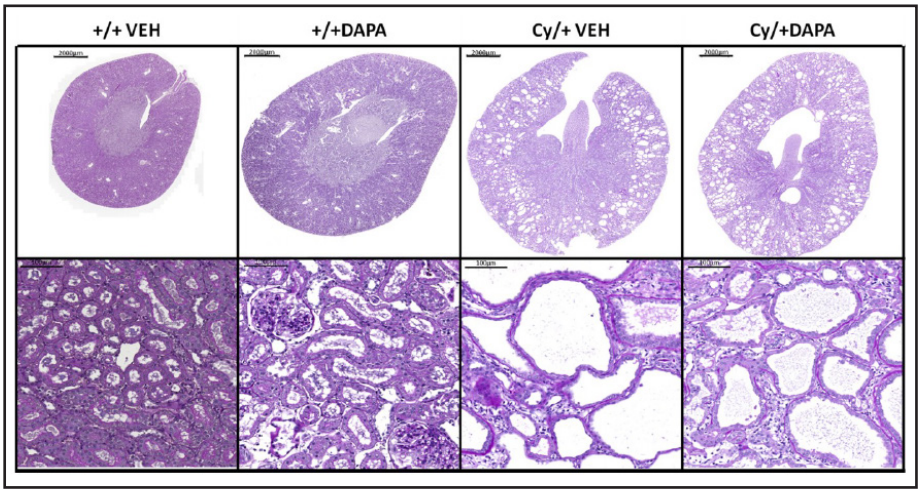


Fig. 1. PAS staining of kidney sections. Sections are from 10 week old Cy/+ and +/+ rats treated with DAPA or VEH for 5 weeks; scale bar is 2000 µm in upper images and 100 µm in lower images.

DAPA increased kidney weight without effect on cyst growth

In +/+ and Cy/+ rats, treatment with DAPA for 5 weeks led to a significant increase in the kidney weight and the 2-kidneys/body weight ratio (2KW/BW) compared to VEH-treated rats (Table 4). The histological examination of the kidneys revealed a slightly higher amount of non-cystic parenchyma but a similar cyst burden in DAPA-treated Cy/+ rats (Fig. 1). Histomorphometric quantification of the cyst burden revealed a similar cyst index in DAPA- and VEH-treated Cy/+ rats (20.3 ± 1.4 vs 21.9 ± 1.2%, $P = 0.55$) (Fig. 2A), a similar total number of cysts (1056 ± 225 vs. 994 ± 229, $P = 0.67$; Fig. 2B) and a similar size profile of the cysts (Fig. 2C). Altogether these data suggests that there was a higher amount of non-cystic tissue in DAPA-treated Cy/+ rats.

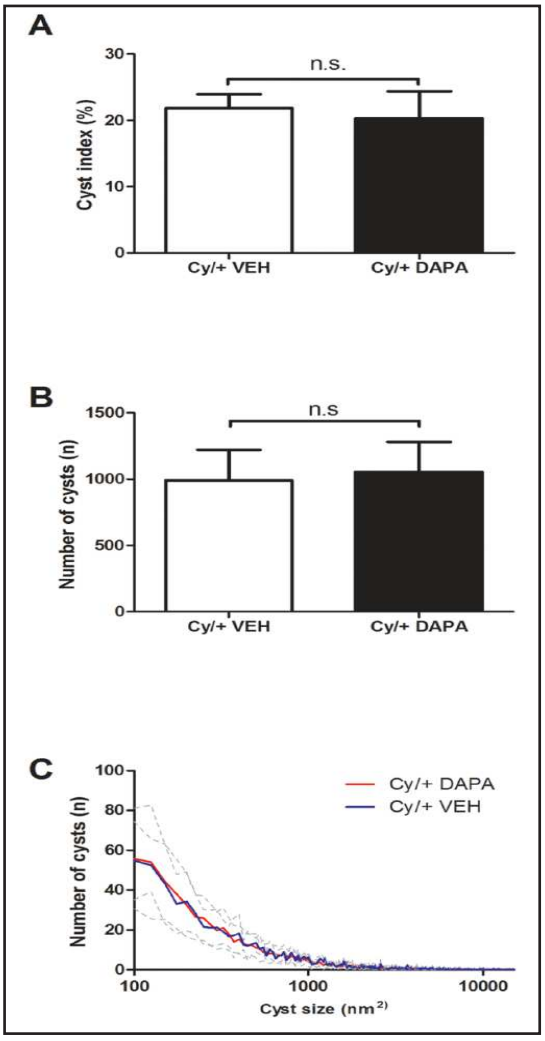


Fig. 2. Histomorphometric analysis of cyst growth. PAS-stained kidney sections of 10 week old Cy/+ rats treated with DAPA or VEH for 5 weeks. (A) Cyst index (%). (B) Number of cysts (n). (C) Cyst classification by size, grey lines represent ranges for ± 1 SD.

DAPA had no effect on renal tubular epithelial cell proliferation

The immunohistological staining for Ki67-positive tubular epithelial cells (TEC) showed that the number of Ki67-positive TECs was higher in Cy/+ than +/+ rats, suggesting enhanced TEC proliferation in Cy/+ rats (Fig. 3). The difference in the number of Ki67-positive TECs between DAPA- and VEH-treated +/+ rats was not significant (139 ± 87 vs. 163 ± 63 cells/mm^2, P = 0.67). DAPA-treated Cy/+ rats exhibited less proliferating TECs than VEH-treated animals (237 ± 109 vs. 327±60 Ki67-positive cells/mm^2), but the difference was also not significant (P=0.28) (Fig. 4A).

DAPA had no effect on renal macrophages infiltration

The degree of infiltration with macrophages was assessed by staining kidney sections for CD68. The number of CD68-positive cells was significantly higher in Cy/+ compared to +/+ rats (20 ± 8 vs. 11 ± 6 CD68-positive cells/mm^2, P = 0.03). However, there was no difference between DAPA- and VEH-treated Cy/+ rats (19 ± 8 vs. 20 ± 8 ED-1-positive cells/mm^2, P = 0.85) (Fig. 4B).

DAPA had no effect on interstitial fibrosis

Using Gomori's Blue Trichrome staining we quantified the degree of renal interstitial fibrosis in Cy/+ and +/+ rats. The renal collagen index was slightly higher in Cy/+ vs. +/+ rat kidneys (5.3 ± 2.0 vs. 4.0 ± 0.8%, P = 0.12). DAPA treatment did not significantly change the collagen index in +/+ (2.9 ± 1.7 vs. 4.0 ± 0.8%, P = 0.11) and Cy/+ rats (4.5 ± 1.8 vs. 5.3 ± 2.0%, P = 0.46) (Fig. 4C).

Discussion

Increasing water intake and diuresis was shown to have beneficial effects on the cystic disease progression in different rodent models of PKD [14-17]. The increased water intake suppresses vasopressin and reduces the translocation of aquaporin 2 from intracellular vesicles to the luminal membrane of the collecting duct, leading to increased diuresis [18]. Furthermore, in the case of PKD, inhibiting vasopressin with tolvaptan at the level of the V_2 receptor in the collecting duct also increases diuresis and reduces intrarenal cAMP, thereby inhibiting cyst epithelial cell proliferation via MAP kinase [19]. The pharmacological inhibition of SGLTs leads to glucosuria which is also accompanied by a significant increase in

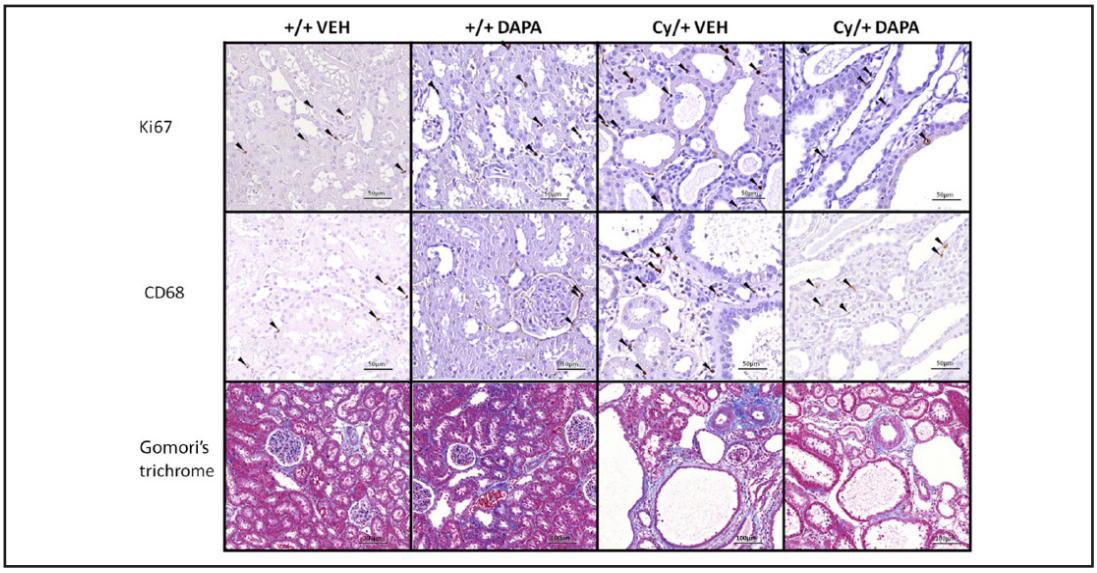
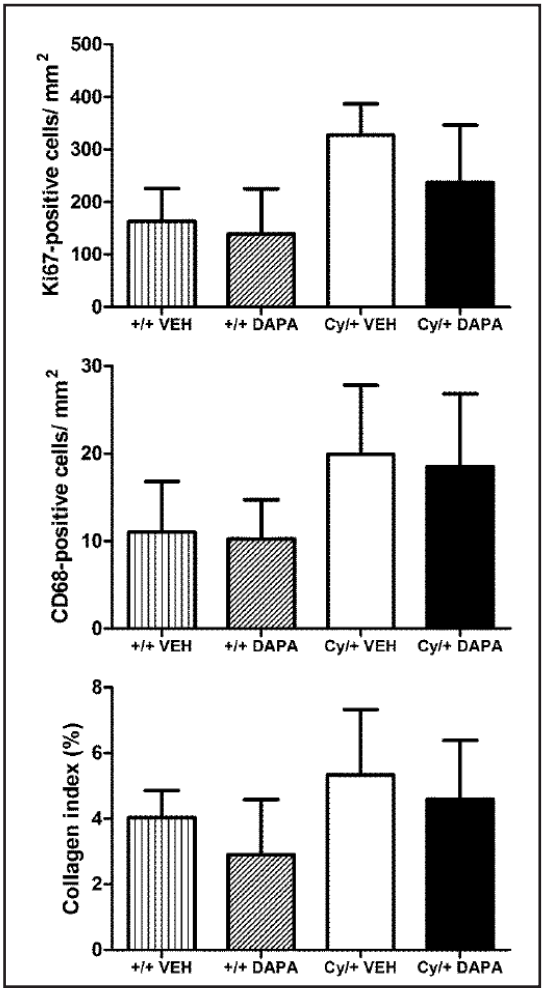


Fig. 3. Histological analysis for Ki67, CD68 and interstitial fibrosis. Kidney sections were analyzed for TEC proliferation (immunostaining for Ki67), macrophage infiltration (immunostaining for CD68) and interstitial fibrosis (Gomori's Blue Trichrome). Arrowheads mark Ki67- or CD68-positive cells. Scale bar is 50 μm for Ki67 and CD68 stainings, and 100 μm in Gomori's Blue Trichrome staining.

Fig. 4. Histomorphometric analysis for Ki67, CD68 and interstitial fibrosis. (A) Ki67 quantification (Ki67-positive cells/mm^2). (B) CD68 quantification (CD68-positive cells/mm^2). (C) Fibrosis quantification (collagen index in %).



diuresis. In analogy to the tolvaptan-induced increase in diuresis which has beneficial effects in PKD we speculated that the increase in diuresis which is caused by SGLT inhibitors could also decrease cyst growth in PKD. Thus, by inducing osmotic diuresis and increasing urinary flow to the collecting duct with the SGLT1/2 inhibitor phlorizin, we recently have shown that cyst growth, renal function and albuminuria improved in the Han:SPRD rat model of PKD [10].

In the present study, we examined the effect of DAPA, a selective SGLT2 inhibitor, on the cystic disease process in Han: SPRD rats. DAPA effectively induced glucosuria and osmotic diuresis but was less potent than the previously tested phlorizin. While DAPA improved the decline of renal function and improved albuminuria it did not decrease cyst growth, macrophage infiltration and interstitial fibrosis in Han: SPRD rats. All in all, the beneficial effects of the SGLT2-selective inhibitor DAPA appeared to be

limited in Cy/+ rats, and are in contrast to the more important effects of the dual SGLT1/2 inhibitor phlorizin.

Of interest, DAPA led an unexpected kidney enlargement which was not due to an increase in the cyst growth. An increase of kidney weight has also been noticed in *Sglt2* -/- knockout mice [20]. Although we do not know the mechanisms behind this increase in kidney weight our histological analysis suggests that it is most likely caused by widening of the tubular lumen due to the increased diuresis. In addition there may also be a certain degree of tubular hypertrophy which could be related to an increase of the SGLT1-mediated glucose reabsorption in the S2 and S3 segment of the proximal tubules [21]. In a different setting, namely in SGLT1-transfected cardiomyocytes a hypertrophic response has also been noticed, suggesting that SGLT1-mediated enhanced glucose uptake may indeed affect cell size [22].

DAPA was developed as a selective SGLT2 inhibitor [23-25] and is in clinical use for the control of glycemia in patients with type 2 diabetes [26, 27]. Since DAPA is also known to increase diuresis [25-28], and given its oral availability and its favorable safety profile [29, 30], we felt that it might be a potentially useful drug to retard disease progression in patients with ADPKD. With DAPA's effect of increasing osmolality along the nephron we speculated that DAPA might also rebalance the transport of fluid across the cyst epithelium, thereby preventing cyst expansion. As mentioned, DAPA was a less effective glucosuric agent than phlorizin and it did not inhibit cyst growth in the Han:SPRD rat model of PKD in the present study, presumably because much of the glucose is reabsorbed by the uninhibited SGLT1 in the proximal tubule [21].

To be effective in PKD, it would be desirable to develop a less selective SGLT inhibitor, displaying strong inhibition of SGLT2 and partial inhibition of SGLT1. This would decrease the SGLT1-mediated glucose reabsorption and enhance the urinary glucose excretion. Ideally such a drug should be orally available, and should not block SGLT1 in the gut completely which might cause glucose-galactose malabsorption and diarrhea [31]. The development of dual SGLT1/2 inhibitors would of course also be predicted to have stronger effects on glycemic control in patients with type 2 diabetes [32].

We recently tested DAPA in PCK rats, an orthologous model of autosomal recessive PKD (ARPKD) [33]. Whereas Han:SPRD rats develop cysts of proximal tubular origin in the cortex, PCK rats develop cysts of distal tubular origin in the medulla. Quite unexpectedly, we found that DAPA led to an increase of the cyst growth in PCK rats. Furthermore the DAPA-treated PCK rats displayed hyperfiltration and albuminuria, eventually accelerating the decline in renal function. Although the renal function was improved with DAPA in Han:SPRD rats we did not find evidence for hyperfiltration, particularly since albuminuria was improved. Altogether the data suggest that DAPA has PKD model-specific effects, and that the effect in human ADPKD could be unpredictable.

Conclusion

The SGLT2-selective inhibitor DAPA improved the decline of renal function and the extent of albuminuria in Han:SPRD rats with PKD, but it did not have a significant effect on cyst growth and secondary renal parenchymal alterations such as macrophage infiltration and fibrosis. Dual SGLT1/2 inhibitors with a stronger glucosuric effect are under development and should be considered for future testing in PKD.

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Disclosure Statement

The authors declare that they have no competing financial interest in the work presented here.

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